Arbuscular mycorrhizal fungi biodiversity: prospecting in Southern-Central zone of Chile. A review

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Abstract

The main factors affecting the diversity of arbuscular mycorrhizal fungi (AMF) are host plant, environmental characteristics and geographical location. A better understanding of the influence of land use changes on AMF abundance will help in improving their management, leading to improved plant productivity in soils, and will also be useful in designing more sustainable agronomic management practices. The aim of this work was to document the occurrence and diversity of AMF species in the Southern Central zone of Chile. This information has been generated by seven separate studies which provide the basis for discussion of the AMF species found in this zone. The work includes a bibliographic review of the records from croplands, grasslands and forests generated by collections made during the period 2004-2014 in Chile. Overall we recorded 21 genera and 66 species of Glomeromycota. This represents 24% of species of AMF known so far. *Acaulospora* represents 23% of all species and *Glomus* 20%. It is concluded that more studies are needed in this and other regions of Chile for a more comprehensive knowledge of the AMF diversity in the country. These biodiversity studies will help to define the ecology of these important soil microbiological resources.

Keywords: Glomeromycota, richness, Shannon-Wiener index, agro-ecosystem, taxonomy

1. Introduction

Arbuscular mycorrhizal fungi (AMF) are thought to be the oldest group of organisms living in symbiosis with land plants (Redecker *et al.*, 2000). AMF form a key functional group of soil biota that can contribute substantially to ecosystems sustainability and plant yields in crop production strategies (Urcoviche *et al.*, 2014). They are beneficial for the whole plant community because they take up inorganic nutrients, e.g. phosphorus (P) and nitrogen (N), and water from the soil; and transport them to the colonized plant roots. In exchange their hosts offer protection and supply the AMF with photo-assimilates (Augé, 2001).

Abiotic factors (e.g. climate, edaphic properties), biotic factors (e.g. dominant vegetation type, interspecific interactions) and intrinsic properties of arbuscular mycorrhizal (AM) fungal species (e.g. dispersal capabilities, extinction rates) influence the abundance and distribution of AMF across multiple spatial scales (Chaudhary *et al.*, 2014). Recent work examined the factors that influence Glomeromycota species distributions at global scales (Turrini and Giovannetti, 2012). Conventional spore-based investigations provide evidence that the distribution of AM fungal spores in soils can be affected by biotic and abiotic factors and soil disturbance regimes (Boddington and Dodd, 2000; Egerton-Warburton and Allen, 2000; Carvalho *et al.*, 2003). Although the community structure of AMF can be inferred from spore communities, it should not be assumed that the AM fungal species inside plant roots, determined using DNA-based techniques, will exhibit the same patterns of occurrence as the spore communities in the surrounding soil (Liu *et al.*, 2012).

The phenology of AMF may generate distinct root and spore communities, and this may help to partition fungal niches in space and time (Pringle and Bever, 2002).

Occurrence of certain AM fungal species has been related to soil pH, P level, salinity, soil disturbance (Abbott and Robson, 1991), vegetation (Johnson *et al.*, 1992), or the hydrological condition of the soil (Miller and Bever, 1999). In general terms, increase in soil pH, nutrient status and salinity could result in a decrease of AM root colonization and spore density (Abbott and Robson, 1991). Despite the importance of AMF in the physiology and nutrition of plants, as well as in shaping plant communities, factors affecting the presence, diversity, spore density, and root colonization of AMF in soil are not well understood (van der Heijden *et al.*, 2006). The main reason is the difficulty of establishing a causal relation between soil and plants factors and AM fungal populations (Vyas and Gupta, 2014). AMF can associate with a wide range of hosts present in the plant community, but the sporulation rates of AMF have been found to be host-dependent (Lugo and Cabello, 2002). Host-dependence of AM fungal populations may play an important role in the maintenance of AM fungal species diversity in grasslands (Bever *et al.*, 1996), and suppression of mycorrhizal symbioses can decrease the dominance of certain plants in a natural ecosystem, thus increasing AMF species diversity (Hartnett and Wilson, 1999). Also, plant diversity may increase or decrease if the dominant plant competitors are weaker or stronger in mycotrophic terms than their neighbours (Hartnett and Wilson, 1999).

Studies conducted at the local scale have suggested that the taxonomic composition of AMF and the dominant AM fungal taxa colonizing plant roots may differ between habitat types, host plant species and locations in the root system (Wubet *et al.*, 2004).

The easiest way to understand in which ecological conditions particular AMF species can be expected, is probably by studying the fungal diversity in different

agro-ecosystems and relating their occurrence with soil characteristics, host plant communities, AMF fungal communities and climatic conditions. With such data available, the bio-geographical distribution can be superimposed, and the ecology of specific AMF can be identified.

2. Glomeromycota phylum: taxonomy and classification

The phylum Glomeromycota was established by Schüssler *et al.* (2001). The phylum is divided into

three classes: *Archaeosporomycetes*, *Glomeromycetes*, and *Paraglomeromycetes*, with five orders: *Glomerales*, *Diversisporales*, *Gigasporales*, *Archaeosporales*, and *Paraglomerales* (Table 1a and 1b). Fifteen families, with 38 genera and about 270 species are known to date (Oehl *et al.*, 2011a; 2015; Goto *et al.*, 2012; Blaszkowski *et al.*, 2013; 2015; Marinho *et al.*, 2014; Silva *et al.*, 2014; and the site <http://www.agro-scope.admin.ch/>).

Table 1a. Taxa of Glomeromycota from class to genus level (Oehl *et al.*, 2011a; Sieverding *et al.*, 2014).

Class	Order	Family	Genus
<i>Glomeromycetes</i> (Cavalier-Smith)	<i>Glomerales</i> (J.B. Morton & Benny)	<i>Glomeraceae</i> Piroz. & Dalpé	<i>Dominkia</i> Blaszk., Chwat & Kovács <i>Funnelformis</i> C. Walker & A. Schüssler <i>Glomus</i> Tul. & C. Tul <i>Kamienskia</i> Blaszk., Chwat & Kovács <i>Rhizoglomus</i> Sieverd., G.A. Silva & Oehl <i>Sclerocystis</i> Berk. & Broome <i>Septoglomus</i> Sieverd., G.A. Silva & Oehl <i>Simiglomus</i> Sieverd., G.A. Silva & Oehl
		<i>Entrophosporaceae</i> Oehl & Sieverd.	<i>Albahypha</i> Oehl, G.A. Silva, B.T. Goto & Sieverd. <i>Claroideoglomus</i> C. Walker & A. Schüssler <i>Entrophospora</i> R.N. Ames & R.W. Schneid <i>Viscospora</i> Sieverd., G.A. Silva & Oehl <i>Corymbiglomus</i> Blaszk. & Chwat <i>Diversispora</i> C. Walker & A. Schüssler <i>Otospora</i> Oehl, Palenz. & N. Ferrol <i>Redeckera</i> C. Walker & A. Schüssler <i>Tricispora</i> Oehl, Sieverd., G.A. Silva & Palenz.
	<i>Diversisporales</i> C. Walker & A. Schüssler	<i>Diversisporaceae</i> (C. Walker & A. Schüssler)	<i>Sacculosporaceae</i> Oehl, Sieverd., G.A. Silva, B.T. Goto, I.C. Sanchez & Palenz. <i>Pacisporaceae</i> C. Walker, Blaszk. A. Schüssler & Schwarzott)
			<i>Sacculospora</i> Oehl, Sieverd., G.A. Silva, B.T. Goto, Sánchez-Castro & Palenz. <i>Pacispora</i> Omehl & Sieverd.

Table 1b. Taxa of Glomeromycota from class to genus level (Oehl *et al.*, 2011a; Sieverding *et al.*, 2014).

		<i>Acaulosporaceae</i> J.B. Morton & Benny	<i>Acaulospora</i> Sieverd., G.A. Silva, B.T. Goto & Oehl <i>Kuklospora</i> Oehl & Sieverd. <i>Bulbospora</i> Oehl & G.A. Silva <i>Orbispora</i> Oehl, G.A. Silva & D.K. Silva <i>Scutellospora</i> C. Walker & F.E. Sanders <i>Dentiscutata</i> Sieverd., F.A. Souza, & Oehl <i>Fuscutata</i> Oehl, F.A. Souza, L.C. Maia & Sieverd. <i>Quatunica</i> F.A. Souza, Oehl, Sieverd. <i>Cetraspora</i> Oehl, F.A. Souza & Sieverd. <i>Racocetra</i> Oehl, F.A. Souza & Sieverd. <i>Intraornatosporaceae</i> B.T. Goto & Oehl <i>Paradentiscutataceae</i> B.T. Goto, Oehl & G.A. Silva <i>Gigasporaceae</i> J.B. Morton & Benny	<i>Acaulospora</i> Sieverd., G.A. Silva, B.T. Goto & Oehl <i>Kuklospora</i> Oehl & Sieverd. <i>Bulbospora</i> Oehl & G.A. Silva <i>Orbispora</i> Oehl, G.A. Silva & D.K. Silva <i>Scutellospora</i> C. Walker & F.E. Sanders <i>Dentiscutata</i> Sieverd., F.A. Souza, & Oehl <i>Fuscutata</i> Oehl, F.A. Souza, L.C. Maia & Sieverd. <i>Quatunica</i> F.A. Souza, Oehl, Sieverd. <i>Cetraspora</i> Oehl, F.A. Souza & Sieverd. <i>Racocetra</i> Oehl, F.A. Souza & Sieverd. <i>Intraornatospora</i> B.T. Goto, Oehl & G.A. Silva <i>Paradentiscutata</i> B.T. Goto, Oehl & G.A. Silva <i>Gigaspora</i> Gerd. & Trappe
<i>Archaeosporomycetes</i> Sieverd., G.A. Silva, B.T. Goto & Oehl	<i>Archaeosporales</i> C. Walker & A. Schüssler	<i>Ambisporaceae</i> C. Walker, Vestberg & A. Schüssler <i>Archaeosporaceae</i> J.B. Morton & D. Redecker	<i>Ambispora</i> C. Walker, Vestberg & A. Schüssler <i>Archaeospora</i> J.B. Morton & D. Redecker <i>Intraspora</i> Oehl & Sieverd. <i>Palaeospora</i> Oehl, Palenz, Sánchez-Castro & G.A. Silva <i>Geosiphon</i> F. Wettst. <i>Paraglomus</i> J.B. Morton & D. Redecker	
<i>Paraglomeromycetes</i> Oehl, G.A. Silva, B.T. Goto & Sieverd.	<i>Paraglomerales</i> C. Walker & A. Schüssler	<i>Paraglomeraceae</i> J.B. Morton & D. Redecker		

Table 1a and 1b shows a schematic presentation of recently published taxa in the Glomeromycota phylum. According to Oehl *et al.* (2011), this classification was based on molecular (SSU, ITS, partial LSU rDNA, and partial b-tubuline gene) and morphological analyses (spore wall structures, structures of the spore bases and subtending hyphae, germination and germination shield structures).

Identification of AMF spores isolated directly from the field or from trap AMF cultures (e.g. Oehl *et al.*, 2005) have been the main sources for the detection and identification of AMF (Robinson-Boyer *et al.*, 2009). Since the first application of nuclear ribosomal gene sequencing to AMF spores (Simon *et al.*, 1992), and root samples from the field (so-called environmental samples) (Helgason *et al.*, 1998), molecular methods have become tools

for studying the natural diversity of AMF (Öpik *et al.*, 2014). However, naturally occurring AMF detected by molecular means still require identification, as the environmental sequences cannot be related to AMF species (Öpik *et al.*, 2014).

3. Arbuscular mycorrhizal fungal diversity

Recording the occurrence of AMF populations in different ecosystems represents the first step toward understanding their functional diversity (Souza *et al.*, 2010). This will enable the application of these species to be developed, particularly for the recovery of degraded soils. Intensive soil ploughing under the conventional cropping system negatively affects the AMF community, decreasing the number of species, and reducing the sustainability of this system when compared to non-tillage (Borie *et al.*, 2006; Borriello *et al.*, 2012). All ecological studies indicate that the frequency of AMF species decreases with land use intensification (Oehl *et al.*, 2003; Tchabi *et al.*, 2008). This decrease in diversity is attributed to a complex selective pressure of agricultural practices, such as ploughing, fertilization and fungicide application (Roldán *et al.*, 2007) and also may be related to the fact that the richness of plant species is reduced by increasing land use and increasing the P nutrient status of the soil. Besides agro-ecosystems, plant species, soil types, weeds, and previous crops also have an effect on the AMF population (Vestberg *et al.*, 2005). Lower AMF species richness has been found in arable fields, while species-rich AMF communities were detected in different natural ecosystems and perennial communities such as tropical forest (Snoeck *et al.*, 2010). Studies from the tropics indicate that the AMF species richness in a monoculture like sugarcane grown in fertile soils (Colombian Mollisols) can be high (Sieverding, 1991). Information about

species composition of AMF communities appears important in understanding mycorrhizal function in ecosystems (Johnson and Pfleger, 1992). Others authors, e.g. van der Heijden *et al.* (1998), have found that plant biodiversity, nutrient capture, and productivity in macrocosms increase significantly with an increase of AM hyphal length and AMF species richness.

Mycorrhizal fungi are of high value for ecosystem functioning and sustainability. More appropriate management of mycorrhizae in poor soils would allow a substantial reduction in the amount of minerals used without losses in productivity, while at the same time permitting more sustainable production management. Studies of AMF species diversity and their functions in different types of land use are crucial for understanding the impact of land use changes on ecosystem services (Soka and Ritchie, 2015).

4. Isolation of AMF species

AMF can be identified either by the morphology of their spores or by PCR-based molecular methods, but it is difficult to compare results obtained by different methods in order to estimate AMF diversity (Sasvári and Posta, 2010). Both methods have some intrinsic weaknesses (Sanders, 2004). Most of the studies have relied on spore morphology for AMF identification; however, spore production does not always reflect the abundance of mycorrhizal species in roots (Clapp *et al.*, 1995). To use the morphological method, the AMF species must have sporulated, and sporulation may be affected by environmental conditions. Also, spores from field samples are difficult to identify. To overcome the problem of 'none-sporulating' periods, spore sampling from ecosystems must be repeated over time and trap or bait pot cultures with soil from the

agro-ecosystems must be established and monitored for the occurrence of spores of specific AMF. However, trap cultures might act as a filter allowing sporulation of only part of the indigenous AMF species which are aggressive enough to colonize and sporulate in a fast-growing host under artificial conditions and in a short time span. Sanders (2004) demonstrated that a taxonomy based solely on morphological characters is influenced by many factors and may not correctly estimate the number of AMF species present due to damaged spores as well as the low sporulation of some species.

Molecular methods may also have problems, firstly because a DNA sequence of a particular gene must be known and correctly identified; the biggest problem, however, is that the genetic variation within a fungal species is high, which may be related to the fact that spores of AMF species may contain several hundred nuclei. According to Sasvári and Posta (2010) using molecular tools to estimate the active colonization of roots by AMF, could give real information about mycorrhizal populations.

5. Ecological groups of arbuscular mycorrhizal fungi

It has been shown that AMF community composition depends on host plant species which may have varying degrees of selectivity among AMF species ranging from selective specialists to non-selective. Oehl *et al.* (2003) were able to group the fungal species into ecological groups. They identified so-called AMF ‘generalists’ which occur under a wide range of land use systems and others which are ‘specialists’ which occur only on e.g. permanent grasslands.

Some of the generalists are of the genus *Glomus*, which includes the most widely distributed AMF species in many ecosystems (Sýkorová *et al.*, 2007), such as *Gl. diaphanum*, and *Gl. fasciculatum* (now *Rhizoglomus fasciculatum*), together with *Funneliformes mosseae* and *Fu. geosporum*, *Claroideoglomus claroideum* and *Septoglomus constrictum*. ‘Specialists’ in the sense that they occur in specific ecosystems such as permanent grasslands include some sporocarp-building *Glomus* and *Sclerocystis* spp. e.g. *Gl. mertonii*. Some species only or preferentially occur under specific acid (*Kuklospora colombiana*) or more neutral to alkaline soil conditions (*Fu. mosseae*, *Gl. badium*) as indicated by Sieverding (1991) and Oehl *et al.* (2011). It is obvious from the literature on mycorrhiza that some of these generalists are reported from all parts of the world, i.e. from tropical as well as temperate zones, dry and wet climates, infertile and fertile soils, low pH and higher pH, sandy and clay soils, temporarily-flooded and never-flooded soils, natural and intensively managed ecosystems. This indicates that at least these so-called ‘generalists’ have been able to invade most existing ecosystems and establish a permanent presence. Other (specialist) species need specific ecological conditions to survive and it appears that many AMF species have a low fitness in intensively managed agro-systems (Oehl *et al.*, 2003).

Gigaspora spp. are frequently developed from spores (Hart and Reader, 2002) and are less common in disturbed agro-ecosystems (Siqueira *et al.*, 1989); they are more common in tropical soils with *Sclerocystis* spp. *Acaulospora* species also seem to be better adapted to soils with pH lower than 5.0 (Siqueira *et al.*, 1984; Bagyaraj, 2014). According to Oehl *et al.* (2004) there is a pattern of succession

in AMF sporulation: *Glomus* species are the first to sporulate while, *Acaulospora* species do so after the winter period. *Scutellospora* species sporulate only in autumn, as does *Gigaspora* species. Previous studies have found members of the Gigasporaceae family, which includes *Scutellospora*, to be more prevalent in sandy soils (Cuenca and Lovera, 2010; Chaudhary *et al.*, 2014).

The abundance and diversity of AMF are favored by low-input agriculture incorporating planting on raised beds and management practices such as zero tillage that do not disturb the physical properties of soil. Conventional agricultural practices cause loss of AM fungal biodiversity and also selectively favour smaller spores. Strong selection pressure favours sporulation of a certain group of AMF, especially species belonging to genera of the Gigasporaceae family.

At present, little is known about the diversity control of AM fungal communities in some regions; given the increasing importance attached to mycorrhizal fungal diversity for the maintenance of ecosystem functioning, a better understanding of the causes of AMF diversity and its loss is necessary. A better understanding of the influence of land use changes on AMF abundance will help in improving their management, thus leading to improved plant productivity in poor soils; it will also be useful for designing more sustainable management practices (Soka and Ritchie, 2015).

6. Indicators used to evaluate AMF diversity

Soils usually contain more than one species of AMF (Barea *et al.*, 2005). In a single rhizosphere,

it is possible to find six to eight different morphotypes (Miranda, 2008). A study conducted by Miranda *et al.* (2005), confirmed that the species richness of AMF is influenced by the associated crop and the type of soil management. The presence of legumes and grasses was associated with an increase in the number of genera and species of AMF over the extended growing season (Cardozo Júnior *et al.*, 2012).

The Shannon diversity index estimates species richness and evenness, and is moderately sensitive to sample size (Zak and Willig, 2004); it takes into account both the number and the relative proportions of taxa in a community. It can range from 0.45 for the AM fungal community in an arable field (Daniell *et al.*, 2001) to 3.0 in a forest habitat (Becerra *et al.*, 2011). According to Brower and Zar (1984), a community with high diversity is characterized by low dominance (few species dominate the AMF community). The Pielou evenness index is used to verify that the distribution among AMF species is uniform.

On the other hand, Wu *et al.* (2007) found that plant species correlated positively with the abundance of AMF, and Picone (2000) demonstrated that replacement of primary forest by pasture does not create differences in the diversity or dominance of mycorrhizal communities.

The biodiversity index often shows a higher correlation with richness than evenness. Species richness can vary between samples due to numerous ecological variables, such as seasonality and host. Indicators used to evaluate AM fungal communities in different ecosystems are shown in Table 2.

Table 2. Arbuscular mycorrhizal fungi diversity index (Moreno, 2001).

Parameter	Formulae
Abundance (n_i)	Number of spores per species i
Relative abundance (RA)	$RA = \frac{\text{N}^\circ \text{ of spores for the species (genus)}}{\text{Total N}^\circ \text{ of spores identified}} \times 100$
Isolation frequency (IF)	$IF = \frac{\text{N}^\circ \text{ soil samples where the species (genus) occurred}}{\text{Total N}^\circ \text{ of soil samples}} \times 100$
Shannon-Wiener diversity index (H')	$H' = - \sum P_i \ln (P_i) *$
Pielou's evenness (J')	$J' = H' / \log(S)**$
Simpson dominance index (I)	$I = \sum [n_i (n_i - 1)] / [N (N - 1)]$
Simpson diversity index (D_s)	$D_s = 1 - I$

* $P_i = n_i/N$, where n_i = number of individuals of the species i ; N = total number of individuals all species (Shannon and Weaver, 1949). ** H' = value obtained by Shannon index, where (S) = total number of species (genera) identified (Pielou, 1975).

Within AM-dominated biomes the composition of AM fungal communities may differ between regions and habitat types. In addition, there were evident differences between ecosystems under different disturbance regimes (Öpik *et al.*, 2006). For example, a low AMF species richness (only 5) was found in the rhizospheric soils associated to pioneer plant species growing at the mouth of lake Budi, Chile (Medina *et al.*, 2015); nevertheless, one of these corresponds to a new species *Corymbiglomus pacificum* (Medina *et al.*, 2014). On the contrary, enormous species richness was found in organic tomato in a Mediterranean site with 58 AMF species belonging to 14 genera (Njeru *et al.*, 2015). Maurer *et al.* (2014) reported 38 AMF species under no-till and in temperate grasslands Oehl *et al.* (2010) found between 34-39 AMF species and Saito *et al.* (2004) in Japan reported least amount with 24 taxa at two sites. In forest, the highest taxon richness has been reported in tropical Atlantic Forests with 30 species (Pereira *et al.*, 2014) and tropical forests in Panamá with 29 taxa at three sites (Husband *et al.*, 2002), not unusual in tropical forests. Only one AM fungal taxon was involved in the strictly specific symbiotic relationships

with non-photosynthetic plants and liverworts (Russell and Bulman, 2005).

In Chile there are a few reports on AMF diversity in different environments performed since 2004. The aim of this article is to make the distribution of Glomeromycota species in the Southern-Central zone of Chile available to science.

7. Bibliographical survey of the recorded species in the study area of Southern-Central zone of Chile

Published descriptions of AMF species were used as the basis for this study. These included the review by Aguilera *et al.* (2014), together with data provided by Montero Sommerfeld *et al.* (2013) and Castillo *et al.* (2004; 2006a; 2006b; 2008; 2010) corresponding to 18 localities. These studies on AMF diversity were grouped into four ecosystem types: forest, grassland, horticultural and crop agro-ecosystem (Table 3). Soil characteristics (pH, organic matter and available-P) of the ecosystems of the Southern-Central zone of the country in Table 4.

Table 3. Site description and geographical position of the ecosystems of the Southern-Central zone of Chile.

Ecosystem symbol	Geographical location	Host	Description
EF	39°30'-39°38'S; 72°02'-72°09'W	<i>N. dombeyi-L. philippiana</i> ; <i>S. conspicua</i>	Evergreen primary forest
DF	39°30'-39°38'S; 72°02'-72°09'W	<i>N. alpina</i>	Secondary forest
GF	39°30'-39°38'S; 72°02'-72°09'W	Natural grassland	Natural grassland
NO	38°46'S; 72°38'W	Natural grassland	Natural grassland after lupine with organic fertilization
N	38°46'S; 72°38'W	Natural grassland	Natural grassland without fertilization
GA	39°47'S; 73°12'W	Grassland	Four grassland sites with different long-term management histories
An	37°48'S; 73°41'W	<i>C. annuum</i>	Pepper plantation
Lo	38°39'S; 73°29'W	-	Fallow after <i>L. sativa</i>
Lu	38°40'S; 73°58'W	<i>C. annuum</i>	Pepper plantation
Pr	38°09'S; 73°00'W	<i>C. annuum</i>	Pepper plantation
Sc	38°15'S; 72°50'W	<i>C. annuum</i>	Pepper plantation
VI	38°40'S; 72°14'W	<i>L. sculentum</i>	Tomato plantation
NT	38°40'S; 72°31'W	<i>T. aestivum</i>	Oat-wheat rotation with no tillage
CT	38°40'S; 72°31'W	<i>T. aestivum</i>	Oat-wheat rotation with conventional tillage
Pu	38°40'S; 72°31'W	<i>T. aestivum</i>	Oat-wheat rotation with no tillage and conventional tillage
Hu	-	<i>T. aestivum</i>	Oat-wheat rotation
EC	-	<i>T. aestivum</i>	Lupine-wheat rotation
Al	-	<i>T. aestivum</i>	Assays in field and trap cultures
Go	-	<i>T. aestivum</i>	Field
Cu	-	<i>T. aestivum</i>	Field
OA	38°34'S; 72°27'W	<i>Avena sativa</i>	Potato-oat in Andisol in pot
LA	38°34'S; 72°27'W	<i>Lupinus</i> sp.	Potato-lupine in Andisol in pot
RA	38°34'S; 72°27'W	<i>Brassica napus</i>	Potato-rape in Andisol in pot
OI	38°02'S; 73°06'W	<i>Avena sativa</i>	Natural grassland-oat in Inceptisol in pot
LI	38°02'S; 73°06'W	<i>Lupinus</i> sp.	Natural grassland-lupine in Inceptisol in pot
RI	38°02'S; 72°06'W	<i>Brassica napus</i>	Natural grassland-rape in Inceptisol in pot

Table 4. Soil characteristics of the ecosystems of Southern-Central zone of Chile.

Ecosystem	Symbol	Location	Type of soil	pH	MO (%)	P (mg kg ⁻¹)
Forest	EF	Panguipulli	Andisol	4.6	22	6
	DF	Panguipulli	Andisol	5.4	25	4
Grassland	GF	Panguipulli	Andisol	5.4	15	3
	NO	Metrengo	Ultisol	5.8-6.1	11-14	21-17
	N	Metrengo	Ultisol	5.7	10	12
	GA	Valdivia	Andisol	5.7-5.8	5-14	4-14
Horticultural	An	Angol	Ultisol	7.3	7	106
	Lo	Lobería	Inceptisol	6.1	2	4
	Lu	Lumaco	Inceptisol	5.9	6	38
	Pr	Purén	Ultisol	5.9	5	48
	Sc	Los Sauces	Ultisol	5.5	6	10
	VI	Vilcún	Andisol	6.1	8	6
	NT	Pumalal	Ultisol	5.8	8	18
Wheat rotation	CT	Pumalal	Ultisol	5.8	8	22
	Pu	Pumalal	Ultisol	-	-	-
	Hu	Huichahue	Andisol	5.4	16	14
Wheat	EC	El Carmen	Ultisol	5.3	6	4
	Al	-	Andisol	4.5	12	-
	Go	Gorbea	Andisol	-	-	-
	Cu	Curacautín	Andisol	-	-	-
	OA	Lautaro	Andisol	6.0	-	6
Others crops	LA	Lautaro	Andisol	5.9	-	4
	RA	Lautaro	Andisol	6.0	-	8
	OI	Lumaco	Inceptisol	5.6	-	2
	LI	Lumaco	Inceptisol	5.3	-	1
	RI	Lumaco	Inceptisol	5.5	-	5

8. Arbuscular mycorrhizal fungal species identification

Morphological: Spores were extracted from 20 g of soil sampled from the study sites and from trap cultures using the wet sieving technique (Gerdemann and Nicolson, 1963) followed by centrifugation in water and sucrose at 70%. Briefly, AMF spores were passed through sieves of 425, 250, 106, 53 and 32 µm and thoroughly washed with distilled water, then separated into morphotypes and mounted on slides using polyvinyl alcohol in lactoglyc-

erol (PVLG) and PVLG + Melzer reagent (1:1 v/v) for species identification. Identification was by micromorphological analysis, using the descriptions of AMF found in Schenck and Pérez (1990) and Błaszkowski (2012), and the sites <http://www.agro.ar.szczezin.pl/jblaszkowski> and <http://invam.caf.wvu.edu>.

Terminal-restriction fragment length polymorphism and sequencing analyses. Plant root DNA was extracted using cetyltrimethyl ammonium bromide (CTAB) according to a modified protocol of Allen *et al.* (2006) as described in Krüger *et al.*

(2009), and the methodology applied by Montero Sommerfeld *et al.* (2013). Electropherograms of the sequenced colonies were compared against those in the public databases by BLASTN searches (<http://www.ncbi.nlm.nih.gov>; Altschul *et al.*, 1997).

9. Diversity of Glomeromycotean species in the study area

In this study covering the Southern-Central zone of the country (Table 3), a total of 21 genera were

found, represented by 57 species of AMF that have been recognized during the last 10 years, equivalent to 21% of all AMF species described worldwide (Table 5).

The number of different AMF species found ranged from 5 to 29 in specific ecosystems. Four species occurred in 9 to 15 of the 20 locations: *Cl. etunicatum* (75%), *Ar. trappei* (50%), *Cl. claroideum* (45%) were found in all the four ecosystems studied. For our country this species could be described as a “generalist”. *Rhizoglosum intraradices* (45%) on the other hand was only found in the agro-ecosystems.

Table 5a. Classification and detection sites of arbuscular mycorrhizal fungal species around of the Southern-Central zone of Chile.

AMF specie	Ecosystem																			
	Forest		Grassland				Horticultural						Wheat rotation				Wheat			
	EF	DF	GF	NO	N	GA	An	Lo	Lu	Pr	Sc	VI	NT	CT	Pu	Hu	EC	Al	Go	Cu
<i>Acaulospora</i>																				
<i>Ac. alpina</i> Oehl, Sýkorová & Sieverd.	X	X	X						X			X								
<i>Ac. cavernata</i> Blaszk.			X			X														
<i>Ac. colossica</i> P.A. Schultz, Bever & J.B. Morton	X		X												X					
<i>Ac. dilatata</i> J.B. Morton.	X		X												X					
<i>Ac. koskei</i> Blaszk.	X		X												X					
<i>Ac. laevis</i> Gerd. & Trappe	X		X			X						X			X			X		
<i>Ac. longula</i> Spain & N.C. Schenck		X	X					X				X						X		
<i>Ac. mellea</i> Spain & N.C. Schenck	X	X	X														X			
<i>Ac. nivalis</i> Oehl, Palenz., I.C. Sánchez, Kuss, Sieverd., G.A. Silva.						X														
<i>Ac. paulinae</i> Blaszk.			X							X		X					X			
<i>Ac. punctata</i> Oehl, Palenz., I.C. Sánchez, G.A. Silva, C. Castillo & Sieverd..		X	X																	
<i>Ac. scrobiculata</i> Trappe			X																	
<i>Ac. sieverdingii</i> Oehl, Sýkorová & Blaszk.																		X	X	
<i>Ac. spinosa</i> C. Walker & Trappe	X								X			X		X						
<i>Ac. thomii</i> Blaszk.			X							X			X							
<i>Acaulospora</i> spp.				1		1						1			2		1	1		

Table 5b. Classification and detection sites of arbuscular mycorrhizal fungal species around of the Southern-Central zone of Chile.

AMF specie	Ecosystem																			
	Forest		Grassland				Horticultural						Wheat rotation					Wheat		
	EF	DF	GF	NO	N	GA	An	Lo	Lu	Pr	Sc	VI	NT	CT	Pu	Hu	EC	Al	Go	Cu
Ambispora																				
<i>Am. gerdemannii</i> C. Walker, Vestberg & A. Schüssler																		X		
<i>Ambispora</i> spp.																		1	1	1
Archaeospora																				
<i>Ar. leptoticha</i> J.B. Morton & D. Redecker			X													X				
<i>Ar. myriocarpa</i> Oehl, G.A. Silva, B.T. Goto & Sieverd.																		X	X	
<i>Ar. trappei</i> J.B. Morton & D. Redecker	X		X				X		X		X	X	X			X	X	X		
<i>Ar. undulata</i> Sieverd., G.A. Silva, B.T. Goto & Oehl.									X			X								
<i>Archaeospora</i> spp.		1	1	2											1			1		
Cetraspora																				
<i>Ce. gilmorei</i> Oehl, F.A. de Souza & Sieverd.						X												X		
<i>Cetraspora</i> spp.												1						1		
Claroideoglossus																				
<i>Cl. claroideum</i> C. Walker & A. Schüssler	X	X			X		X		X			X				X	X	X		
<i>Cl. etunicatum</i> C. Walker & A. Schüssler	X	X	X	X			X	X	X		X		X	X	X		X	X	X	X
<i>Cl. lamellosum</i> C. Walker & A. Schüssler																	X			
<i>Di. spurca</i> C. Walker & A. Schüssler	X		X					X			X		X		X		X			

Table 5c. Classification and detection sites of arbuscular mycorrhizal fungal species around of the Southern-Central zone of Chile.

AMF specie	Ecosystem																			
	Forest		Grassland				Horticultural						Wheat rotation					Wheat		
	EF	DF	GF	NO	N	GA	An	Lo	Lu	Pr	Sc	VI	NT	CT	Pu	Hu	EC	Al	Go	Cu
<i>Dominikia</i>																				
<i>Di. epigaea</i> A. Schüssler, Krüger, C. Walker								X	X	X	X	X								
<i>Do. aurea</i>																		X		
<i>Entrophospora</i>																				
<i>En. infrequens</i> R.N. Ames & R.W. Schneid. emend. Oehl & Sieverd.	X	X																		
<i>Funnelformis</i>																				
<i>Fu. coronatus</i> Giovann.									X			X	X	X	X					
<i>Fu. geosporus</i> C. Walker	X		X								X									
<i>Fu. monosporus</i> Gerd. & Trappe												X								
<i>Fu. mosseae</i> C. Walker & A. Schüssler									X		X	X				X	X	X		X
<i>Gigaspora</i>																				
<i>Gigaspora</i> spp.							1										1			
<i>Glomus</i>																				
<i>Gl. ambisporum</i> G.S. Sm. & N.C. Schenck																X				
<i>Gl. brohultii</i> Sieverd.			X																	
<i>Gl. clavisorum</i> Almeida & N.C. Schenck							X													
<i>Gl. diaphanum</i> J.B. Morton & C. Walker			X							X		X	X	X	X			X		
<i>Gl. macrocarpum</i> Tul. & C. Tul.	X	X	X						X				X		X					X
<i>Gl. pallidum</i> I.R. Hall							X	X	X			X								
<i>Gl. vesiculiferum</i> C. Walker & A. Schüssler									X											
<i>Glomus</i> spp.				8	7										2	1	1	2		

Table 5d. Classification and detection sites of arbuscular mycorrhizal fungal species around of the Southern-Central zone of Chile.

AMF specie	Ecosystem																			
	Forest		Grassland				Horticultural						Wheat rotation				Wheat			
	EF	DF	GF	NO	N	GA	An	Lo	Lu	Pr	Sc	VI	NT	CT	Pu	Hu	EC	Al	Go	Cu
<i>Intraspora</i>																				
<i>In. schenkii</i> Sieverd. & S. Toro	X		X												X					
<i>Pacispora</i>																				
<i>Pa. dominikii</i> Oehl & Sieverd.			X					X				X			X		X	X		
<i>Pacispora</i> spp.	2	1															1			
<i>Paraglomus</i>																				
<i>P. occultum</i> J.B. Morton & D. Redeker			X						X		X	X				X		X	X	X
<i>P. laccatum</i> Blaszk.			X																	
<i>Racocetra</i>																				
<i>Ra. weresubiae</i> Koske & C. Walker			X																	
<i>Rhizoglomus</i>																				
<i>R. aggregatum</i> N.C. Schenck & G.S. Sm.												X								
<i>R. fasciculatum</i> C. Walker & Koske		X						X	X			X				X			X	X
<i>R. intraradices</i> N.C. Schenck & G.S. Sm.							X	X		X			X	X	X			X	X	X
<i>R. invernium</i> I.R. Hall	X					X							X	X					X	X
<i>R. microaggregatum</i> Koske, Gemma & Olexia																				X
<i>Sacculospora</i>																				
<i>Sa. baltica</i> Blaszk.			X																	
<i>Sclerocystis</i>																				
<i>Sc. rubiformis</i> Gerd. & Trappe	X	X													X					
<i>Sc. sinuosa</i> Gerd. & Bakshi																				X

Table 5e. Classification and detection sites of arbuscular mycorrhizal fungal species around of the Southern-Central zone of Chile.

AMF specie	Ecosystem																			
	Forest		Grassland					Horticultural					Wheat rotation					Wheat		
	EF	DF	GF	NO	N	GA	An	Lo	Lu	Pr	Sc	VI	NT	CT	Pu	Hu	EC	Al	Go	Cu
<i>Sclerocystis</i> spp.																1				
<i>Scutellospora</i>																				
<i>Sc. auriglobosa</i> C. Walker & R.E. Sanders			X																	
<i>Sc. calospora</i> C. Walker & F.E. Sanders			X										X	X	X			X		X
<i>Sc. dipurpurens</i> J.B. Morton & Koske			X									X	X	X						
<i>Scutellospora</i> spp.															3	1	1			
<i>Septoglomus</i>																				
<i>Se. constrictum</i> Sieverd., G.A. Silva & Oehl																		X		
<i>Simiglomus</i>																				
<i>Si. hoi</i> G.A. Silva, Oehl & Sieverd.																X		X	X	
R	20	14	29	10	8	6	7	8	14	5	7	21	11	8	22	11	14	24	11	9

Others were rare species unique in their ecosystems. In forest: *Sa. baltica*; in grassland: *Ac. nivalis*, *Ac. scrobiculata*, *Gl. brohultii*, *Pa. laccatum*, *Ra. weresubiae*, *Sc. auriglobosa*; in horticultural agro-ecosystems: *Fu. monosporum*, *Gl. clavisorum*, *Gl. vesiculiferum*, *R. aggregatum*, and in crop agro-ecosystems:

Am. gerdemannii, *Do. aurea*, *Gl. ambisporum*, *Rh. microaggregatum*, *Sc. sinuosa*, and *Se. constrictum*. These species are denominated “specialists”. The families with the most species recorded in the Southern-Central zone of the country were *Glomeraceae* (19) and *Acaulosporaceae* (15), followed

by *Archaeosporaceae* (5), *Entrophosporaceae* (4), *Scutellosporaceae* (3), *Diversisporaceae* (2), *Paraglomeraceae* (2) *Racocetraceae* (2), *Sclerocystis* (2), and finally *Ambisporaceae*, *Gigasporaceae*, *Pacisporaceae*, and *Sacculosporaceae* with only one species each (Table 5).

Of the 38 known genera of AMF, 15 were not recorded in this survey: *Albahypha*, *Corymbiglomus*, *Dentiscutata*, *Fuscutata*, *Geosiphon*, *Intraornatospora*, *Kamienskia*, *Kuklospora*, *Paradentiscutata*, *Quatu-*

nica, *Viscospora*, *Orbispora*, *Otospora*, *Redeckera*, and *Tricispora*.

In the two Chilean forest ecosystems (Figure 1A) most species were from *Diversisporales*, followed by *Glomerales* and *Archaeosporales*. In pasture ecosystems (Figure 1B), also most species were from *Diversisporales*, while in the agro-ecosystems (Figures 1C and 1D) *Glomerales* spp. dominated, followed in several locations by *Diversisporales* and in others by *Archaeosporales*.

Figure 1. Arbuscular mycorrhizal fungi orders distribution in ecosystems of the Southern-Central zone of Chile: Forest (A), Grassland (B), Horticultural (C), and Wheat rotation (D). Glo: Glomerales; Div: Diversisporales; Gig: Gigasporales; Arc: Archaeosporales; Par: Paraglomerales.

In three ecosystems (forest, grassland and wheat agro-ecosystems) *Acaulosporaceae* was the predominant family with 27% to 41%; in horticultural agro-eco-

systems, *Glomeraceae* was the predominant family with 38% (Table 6).

Table 6. Distribution of arbuscular mycorrhizal fungi families in ecosystems of the Southern-Central zone of Chile.

Order	Familie	Agroecosystems		Ecosystems	
		Horticultural (%)	Wheat (%)	Grassland (%)	Forest (%)
<i>Glomerales</i>	<i>Glomeraceae</i>	38	27	16	16
	<i>Entrophosporaceae</i>	7	10	6	13
<i>Diversisporales</i>	<i>Diversisporaceae</i>	7	3	3	4
	<i>Sacculosporaceae</i>	0	0	0	4
	<i>Pacisporaceae</i>	4	5	3	8
	<i>Acaulosporaceae</i>	24	27	41	38
<i>Gigasporales</i>	<i>Scutellosporaceae</i>	4	7	6	0
	<i>Racocetraceae</i>	3	5	6	0
	<i>Gigasporaceae</i>	3	2	0	0
<i>Archaeosporales</i>	<i>Ambisporaceae</i>	0	5	0	4
	<i>Archaeosporaceae</i>	7	7	13	13
<i>Paraglomerales</i>	<i>Paraglomeraceae</i>	3	2	6	0

According to the distribution and prospecting of fungi from the Southern-Central zone of Chile, the following biodiversity indices were obtained: natural grassland (GF) showed the highest AMF species richness (29) followed by evergreen forest (EF) (20); while managed prairie (GA) had the lowest richness (6). Pielou's evenness index was similar in different agro-ecosystems; lupine

(LI) (0.93) presented the highest evenness index and wheat (AI) (0.46 to 0.55) the lowest. Overall, Shannon's diversity index was high in all agro-ecosystems (1.8 to 2.6), being less dominant in lupine (LI) (0.09). The highest value of Simpson's index was found in EF (0.29), coinciding with the highest number of spores (3164 spores 100 gss⁻¹) (Table 7).

Table 7. Arbuscular mycorrhizal fungi diversity index in ecosystems of the Southern-Central zone of Chile.

Ecosystem	Symbol	R	E	H'	S	N° spores
Forest	EF	20	0.62	1.82	0.29	3164 ^a
	DF	14	0.70	1.81	0.24	456 ^a
Grassland	GF	29	0.72	2.19	0.15	1001 ^a
	NO	10	0.68-0.80	1.6-1.8	-	840-1200 ^a
	N	8	0.76	1.6	-	640 ^a
	GA	6	-	-	-	-
Wheat rotation	Pu	10-20	0.78-0.88	1.8-2.5	-	150-700 ^b
Wheat	Al	15-19	0.46-0.55	2.08-2.39	0.10-0.19	80-260 ^a
Other crops	OA	33	0.69	2.4	0.18	550 ^b
	LA	23	0.84	2.6	0.09	180 ^b
	RA	23	0.79	2.4	0.11	390 ^b
	OI	22	0.73	2.3	0.15	420 ^b
	LI	13	0.93	2.4	0.09	50 ^b
	RI	11	0.81	2.0	0.18	50 ^b

N° spores: ^aN° 100 gss⁻¹, ^bN° 100 mL soil⁻¹. R: richness; E: evenness; H': Shannon-Wiener; S; Simpson index

A total of 57 AMF species have been detected during the last 10 years in Chilean soils, equivalent to 21% of global diversity; this is an astonishingly low number, when about 270 species have been classified worldwide for the phylum Glomeromycota (Silva *et al.*, 2014). The number of AMF species can reach up to 60-70 in a relatively small region like the Upper Rhine Valley where Germany, France and Switzerland meet (Sieverding and Oehl, 2005).

In agro-ecosystems, some species were recorded in low numbers; the rarity of some species may reflect their narrow adaptability in contrast to several Glomeraceae species, which have proved more adaptable. All the information currently available indicates that *Glomus* spp. is far more frequent than any of the other genera of AM. Schenck and Kinloch (1980) attributed the abundance of *Glomus* species in the soils to their wide adaptability to different plants and environmental conditions, citing the fact that *Gl. intraradices* (now *Rhizoglomus intraradices*) was found in 9 of the 18 agro-ecosystems (horticultural and crop agro-ecosystems); on the other hand it was absent from

forest and grassland. Douds and Schenck (1990) reported that *Gl. intraradices* has been found to be less sensitive to P and N fertilization, suggesting that the intracortical sporulation of *Gl. intraradices* may reduce the path of carbon flux and could contribute to the relative insensitivity of this species to fertilization. While *Acaulospora* spp. are also found regularly, *Scutellospora* spp., *Entrophospora* spp. and *Pacispora* spp. are less frequent. In tropical agro-ecosystems *Acaulospora* spp., *Glomus* spp. and *Scutellospora* spp. may present the same high frequency, and *Entrophospora* spp. and *Gigaspora* spp. are also often observed, while *Pacispora* spp. are extremely rare (Mello *et al.*, 2013).

In forest ecosystems, *Glomus* spp. were present in 19% of the total. The genus *Glomus* is reported to be the dominant AMF in some forest ecosystems (Tamuli and Boruah, 2002). The genus *Glomus* is reported to be the dominant AMF in some forest ecosystems.

The genus *Intraspora* has been found in Brazil, Argentina, and the Swiss Alps (Sieverding and Oehl, 2006; Cardozo-Júnior *et al.*, 2012), while in Chile it was

only found in EF and GF. Souza *et al.* (2010) noted that members of the genus *Intraspora* are often found in pastures.

Similar results, as just mentioned above, were detected when compared with other studies. Data from semiarid and arid areas show that the richness varies from 3 to 32 AMF species (Yang *et al.*, 2010; Alguacil *et al.*, 2012; Pagano *et al.*, 2013). In the evergreen forests and pastures of Nicaragua and Costa Rica, 28 AMF species have been registered (Picone, 2000), whereas in the oak savannahs of USA 18 AMF species were found (Landis *et al.*, 2004). Furthermore, according to Becerra *et al.* (2011) the genus *Acaulospora* contributed most to the biodiversity in a forest in Argentina. Finally, Kumar and Garampalli (2013) suggested that future research on AMF biodiversity in a complex habitat should combine the molecular, morphological and biochemical approaches. So far, this has been done only once (Wetzel *et al.*, 2014).

10. Final considerations and conclusions

These results on the occurrence and distribution of AMF in the Southern-Central zone of the Chile reinforce the importance of inventories and studies in areas which have not yet been explored, in order to extend knowledge concerning this important group of fungi. The genetic variations among AMF species and between individuals of the same species may influence their compatibility with plants and determine their beneficial effects on the host plants. Knowledge of these aspects may help to elucidate the different roles of AMF in several plant species, and in specific environmental conditions of agro-ecosystems and ecosystems.

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